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EFFECT OF SEED SIZE AND DENSITY ON NEAR-INFRARED TRANSMITTANCE ANALYSIS OF CORN AND SOYBEANS

C. R. Hurburgh Jr., Y. Wu, J. Siska

ABSTRACT. Soybean physical and chemical properties changed by size (from 4.8 to 8.8 mm diameter), but soybean size and seed density did not affect the protein and oil determination accuracy of three near-infrared transmission analyzers. Corn samples were also separated by size and kernel density. Changes in corn kernel density and size introduced small errors in near-infrared transmission protein, oil, and starch measurements. In corn protein, the maximum error was about $\pm 0.2\%$ points. A robust calibration set is needed to eliminate the weak seed weight and density effects, as well as to support the corn density calibration for near-infrared analyzers. **Keywords.** Near infrared, Corn, Soybeans, Composition, Density.

Transmission of near-infrared light (800 to 1100 nm) is a rapidly developing method for measuring composition of unground grain. Seed geometry and density are theoretically important determinants of near-infrared transmission (NIT). Seed size and shape affect the path length of transmitted light, and seed density affects the constituent concentration per unit path length. Therefore, both parameters could affect the accuracy of bulk-grain NIT analyses, and neither has been previously researched.

Whole-grain near-infrared, either transmittance or reflectance, is the method of choice for marketplace measurement of grain composition. The Official USDA grain inspection system has successfully used one NIT model for soybean protein and oil analyses (Watson and Bashanti, 1990) and wheat protein analyses (Funk, 1994; FGIS, 1990a). The Danish market has used the same NIT unit for composition measurements in wheat and barley (Buchmann, 1992).

Seed density (specific gravity, as opposed to bulk density) and seed size have been related to near-infrared transmission through single seeds. Orman and Schumann (1992) reported that seed orientation, geometry, and weight did not affect the accuracy of single-seed transmittance predictions of oil in corn. Lamb and Hurburgh (1992) found only minor effects of soybean seed size and density on predictions of moisture when the instrument calibration set explicitly contained seeds of varying size and density. It

was not known whether these conclusions were applicable to bulk sample measurements.

OBJECTIVE

The objective of this work was to determine the potential magnitude of soybean and corn seed size and density effects on the prediction of proximate composition by NIT analyzers.

MATERIALS AND METHODS

SAMPLES – SOYBEANS

Samples of eight soybean varieties (1989 crop, central Iowa) were sized in round-hole increments of 2/64th in. (0.8 mm), beginning at 12/64 in. (4.8 mm). The samples were cleaned over a 10/64- \times 3/4-in. (4- \times 19.2-mm) slot sieve (referred to as 10S) before analysis. All cleanings and sizings were done with a Carter Dockage tester with the target screen always in the top (kicker) rack. Table 1 gives the size distribution of these samples.

A mixed-variety set was also tested. The 1,644 samples returned in the 1991 American Soybean Association survey were cleaned (with the 10S sieve) and combined by state. The state composites were subsampled, with the subsamples sized in the same way as before. State samples that were heavier than 25 kg (55 lb) were probed with a compartmented hand-probe. Smaller samples were either divided (with a Boerner divider) or analyzed as a whole. Table 2 contains the numbers of individual samples per state and the size distribution of composites. The mean size was calculated as the weighted average, by using the midpoint of each size range to represent the diameter in that range.

Table 1. Size distribution of eight single-variety soybean samples

	Percent of Sample Weight, by Size Range			
	4.8-5.6	5.6-6.4	6.4-7.2	7.2-8.0
mm				
64th in.	12- 14	14-16	16-18	18-20
Average	0.4	29.6	64.6	5.4
Maximum	1.2	58.5	76.9	11.5
Minimum	0.0	10.2	40.3	0.0

Article was submitted for publication in august 1994; reviewed and approved for publication by the Food and Process Engineering Inst. of ASAE in March 1995.

Research supported by the Federal Grain Inspection Service and the Iowa Agriculture and Home Economics Experiment Station. Journal Paper J-15964 of the Iowa Agriculture and Home Economics Experiment Station, Project 2339.

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Table 2. Size distribution of the mixed-variety soybean samples from the 1991 ASA survey (by state)

Region	State	n	Percent Size Distribution, by Size Range, in 64th in.* (mm)						Weighted Mean Size (64th in.)*
			(4.0-4.8)	(4.8-5.6)	(5.6-6.4)	(6.4-7.2)	(7.2-8.0)	(8.0-8.8)	
Western Corn Belt	Iowa	287	0.1	2.5	27.8	60.7	8.5	0.4	16.5
	Kans.	32	0.4	12.0	53.9	32.2	1.6	-	15.5
	Minn.	117	0.1	3.2	36.0	55.1	5.6	0.1	16.3
	Mo.	115	0.1	4.7	47.5	45.4	2.3	-	15.9
	Nebr.	95	0.1	4.7	43.8	47.6	3.9	-	16.0
	N.D.	20	0.1	7.2	61.4	30.2	0.5	-	15.5
	S.D.	42	0.2	11.2	64.1	23.9	0.7	-	15.3
		708							
Eastern Corn Belt	Iowa	385	0.1	3.2	37.3	54.0	5.3	0.1	16.1
	Ind.	140	0.1	2.3	32.9	58.8	5.8	-	16.3
	Mich.	30	0.1	1.7	22.9	64.1	11.3	-	16.7
	Ohio	168	0.1	3.3	33.9	55.8	6.9	0.1	16.3
	Wis.	4	-	0.8	16.0	73.1	10.1	-	16.9
		727							
Midsouth	Ark.	50	0.1	6.6	59.1	33.2	1.0	-	15.6
	Ky.	26	-	5.1	54.4	38.9	1.6	-	15.7
	La.	16	0.3	10.1	54.0	26.0	0.7	-	15.2
	Miss.	41	0.1	6.5	61.7	31.0	0.7	-	15.5
	Okla.†	2	-	-	-	-	-	-	-
	Tenn.	16	-	4.0	52.9	41.6	1.5	-	15.8
	Tex.†	2	-	-	-	-	-	-	-
		153							
Southeast	Ala.	6	-	3.8	48.2	46.8	1.1	-	15.9
	Fla.†	2	-	-	-	-	-	-	-
	Ga.	5	0.1	3.1	49.1	46.3	1.4	-	15.9
	N.C.	11	0.1	5.7	61.0	32.8	0.4	-	15.6
	S.C.	3	-	2.7	50.5	46.3	0.1	-	15.8
		27							
East Coast	Del	7	0.1	8.0	63.9	25.5	2.6	-	15.5
	Md.	13	-	4.4	36.9	55.1	3.6	0.1	16.2
	N.J.†	3	-	-	-	-	-	-	-
	Pa.†	1	-	-	-	-	-	-	-
	Va.	5	0.1	9.1	62.9	27.6	0.5	-	15.4
		29							

* Not enough grain to size.

† 0.4 mm = 1/64 in.

The mixed-variety set contained an unknown number of varieties. If a size effect or a density effect is to be compensated, the effects must be consistent in samples of widely diverse origin. Interestingly, the long-held perception in the Asian market that Eastern Corn Belt soybeans are larger than soybeans from the rest of the country was supported.

SAMPLES – CORN

Corn samples, each weighing about 3500 g (7.7 lb), were obtained in spring 1992. Five were collected at local elevators from inbound producer grain. Five were taken from previous years' test-plot samples, stored at 2° C in the Iowa State University Grain Quality Laboratory. Each sample contained only one corn hybrid, and all samples were different.

Samples were cleaned over a 12/64-in. (4.8-mm) round-hole screen (12R screen) placed in the top rack of a Carter Dockage tester. This is the same procedure as used by USDA, Federal Grain Inspection Service (FGIS) to remove broken corn-foreign material (BCFM) (FGIS, 1990b).

LABORATORY PROCEDURE – SINGLE VARIETY SOYBEAN SAMPLES

The sized samples were analyzed for moisture, protein, oil, and fiber content (basis 13.0% moisture) by the

following near-infrared instruments and laboratory tests, in duplicate:

- Laboratory chemical analysis (moisture – ISU, using Federal Grain Inspection Service procedure (FGIS, 1986); protein, oil, fiber – Woodson-Tenant, Inc., Des Moines, Iowa)
- Dickey-john Instalab 800 near-infrared reflectance (NIR) (ground-grain) (ISU 10-filter, multiple-linear calibration S6 using Woodson-Tenant chemistry values) (Dickey-john, Inc., Auburn, Ill.)
- Tecator Infratec 1225 (whole-grain, transmission monochromator; 800 to 1100 nm) (Tecator calibration US22) (Perstorp Analytical, Inc., Silver Spring, Md.)
- Trebor 99 (whole-grain, transmission fixed wavelength; 12 filters) (ISU multiple-linear calibration ST4 using Woodson-Tenant chemistry values) (Trebor, Inc., Gaithersburg, Md.)
- Foss Grainspec (whole-grain, transmission fixed wavelength; 33 filters) (ISU PLS-based calibrations using Woodson-Tenant chemistry values) (Foss Food Technology, Inc., Eden Prairie, Minn.)

The Dickey-john, Trebor, and Foss units were calibrated at Iowa State University, against chemistry data provided by Woodson-Tenant, Inc., Des Moines, Iowa. The Infratec was calibrated by the manufacturer (PAI, 1989). The

Infratec is used by the FGIS of the USDA, but the FGIS calibrations were not used because moisture was not predicted by the FGIS calibrations. The samples in this project were not used in any of the calibration sets.

The following physical tests also were run in duplicate on each sized sample:

- Test weight, lb/bu (FGIS, 1986).
- Density, g/cc, by air-comparison pycnometer (Beckman model 930) (Dorsey-Redding et al., 1989).
- Thousand-seed weight, TGW, g (weight of counted seeds), counted by hand.

At the completion of a set of tests, triplicate whole-seed oven moistures (AOCS, 1987; Hartwig and Hurburgh, 1990) also were run.

LABORATORY PROCEDURE –

MIXED VARIETY SOYBEAN SAMPLES

The sized samples were analyzed by the same tests, except that the Foss Grainspec had been returned to the United Kingdom for optical modification. Ground-grain NIR (Instalab 800) analyses were used in lieu of wet chemistry for original determination of protein and oil.

Subsamples were always prepared by mechanical division. The soybeans had previously been screened over a 10S sieve as a part of another project (Hurburgh, 1994). Nonetheless, a few splits remained. These were removed by hand.

LABORATORY PROCEDURE – CORN

The cleaned samples were weighed, then sized over a 20/64-in. (8-mm) round-hole screen (20R screen), again in the top rack of the Carter Dockage tester. The “throughs” are referred to as thins. This sizing procedure is used by dry millers to estimate size uniformity in corn (Roskens, 1990).

The sized portions (two per sample) were then separated into heavy and light fractions with a Kice DT6 Multiaspirator (Kice Industries, Wichita, Kans.) set at its highest airflow (400 on the indicator dial). Heavies were grains passing through the aspirator; lights were lifted from the stream. Aspiration separates by terminal velocity; in samples of similar geometry and surface texture, terminal velocity is most influenced by density (specific gravity). There were now four portions per sample (large and thin, with heavy and light in each size).

Table 3 gives the percentages for the size and weight distribution of the 10 samples. Overall, the samples were about 50% thins, although there was sizeable sample-to-sample variability. There were always fewer lights than heavies. More vigorous aspiration might have given more lights, but at the expense of density differentiation between lights and heavies. The goal was to have enough lights to analyze, but retain maximum possible density separation.

Each portion was subjected to the following tests, in duplicate:

- Tecator Infratec 1225 [moisture, protein, and oil percentages by the MA4 factory calibrations (PAI, 1988)].
- Dickeyjohn Instalab 800 (moisture, protein, oil, starch, density, by C6 10-filter, multiplelinear calibrations

Table 3. Size and weight* distribution of 10 single-hybrid corn samples

	Percent of Sample Weight					
	Thins through 20/64 in (8.0 mm)			Larger than 20/64 in (8.0 mm)		
	Heavy	Light	Heavy	Light	Heavy	Light
Average	35.6	16.9	52.5	33.9	13.6	47.5
Maximum	59.2	46.5	92.3	53.2	52.1	68.0
Minimum	22.4	9.4	32.0	7.4	0.2	7.7

* As classified by a Kice DT6 laboratory aspirator.

done by Iowa State University with Woodson-Tenant chemistry value).

- Test weight (lb/bu) (FGIS, 1986).
- Kernel density (g/cc) by air pycnometer (Dorsey-Redding et al., 1989).
- Seed weight (g/1000 seeds) as weight of counted seeds.

All corn data was adjusted to 15% moisture with the equations developed by Dorsey-Redding et al. (1989).

STATISTICAL ANALYSIS –

SINGLE VARIETY SOYBEAN SAMPLES

The physical properties of the sized samples were summarized by size and variety, then were correlated with the Woodson-Tenant laboratory measurements of protein, oil, and fiber content. It was expected that larger seeds would contain less fiber, more protein, and perhaps more oil than smaller seeds of the same variety.

Two randomized, complete block (RCBD) analyses of variance were done—one with the 14 to 16 and 16 to 18 sizes only from all eight varieties and one with the four varieties having more than 400 g in three sizes (14 to 16, 16 to 18, and 18 to 20). Maintaining all sizes across varieties was crucial to accurate data interpretation. The analysis variables were protein and oil contents. Treatments were seed size and instrument with varieties (samples) as blocking factors.

STATISTICAL ANALYSIS –

MIXED VARIETY SOYBEAN SAMPLES

The same RCBD analysis of variance was used for these samples as for the single variety samples. States were the blocking factors. Samples collected from seven states had the necessary 250 g samples in four size ranges, while samples from 10 states had sufficient samples in three size ranges.

A correlation matrix included the physical factors, composition by the several units, and composition differences between whole-grain and ground-grain near-infrared analyzers. This matrix was calculated both by size and with all sizes combined.

STATISTICAL ANALYSIS – CORN SAMPLES

Physical and chemical data were averaged by size and weight separation. The analysis of variance used samples as blocking factors, and size (large versus thin) and type (heavy versus light) as treatments, with one replication per sample.

A correlation matrix included the grain properties and the differences between the NIT predicted composition and the NIR predicted composition. Again, this analysis contained the tacit assumption that the ground-grain NIR

test was unaffected by grain physical properties, an assumption based on the previous calibration data. In the original calibration and validation sets for the Instalab NIR instrument, no grain physical property was correlated with differences between chemical methods and NIR-predicted values.

RESULTS AND DISCUSSION

SINGLE-VARIETY SOYBEAN SET

The general properties of the single-variety soybean samples are given in table 4. The data is divided into two groups, the "8-2" group (all eight varieties, two sizes only) and the "4-3" group (four varieties with three sizes represented).

As size increased, protein and oil increased, and fiber decreased. Test weight decreased (packing effect), but there was no difference in seed density. Larger soybeans apparently have higher nutrient levels but lower test weight. Larger beans have less surface area (fibrous hull) to volume and do not pack as closely in the test weight cup.

The analyses of variance for instrument differences are summarized in table 5. The random errors (RMSE's) were remarkably low, showing that the critical parameters for protein and oil testing were indeed included in the model. Varietal differences (V) and instrument differences (I) were expected to be significant and they were. The size (S) effects were explained by the chemistry data. Of most importance to this study were the interaction terms ($I \times V$) and ($I \times S$).

The ($I \times S$) term measures the effect of geometry on instrument performance. Ideally, ($I \times S$) would be non-significant if calibrations are successfully compensating for geometric variations. This term was nonsignificant; across sizes, the instruments tracked each other and the chemistry laboratory.

The significant ($I \times V$) term shows that other factors influence instrument performance. Figure 1 shows the varietal data for protein (on the "4-3" set), with the seed density listed below the bars. There were relative shifts of up to 0.4 percentage points in the position of instruments versus the laboratory and versus each other. This would have the practical consequence of causing intermarket

Table 5. Summary of analyses of variance, single-variety soybean set

Source	d f *		Protein		Oil		Fiber	
	8-2†	4-3‡	8-2	4-3	8-2	4-3	8-2	4-3
Variety (V)	7	3	§	§	§	§	§	§
Instrument (I)	4	4	§	§	§	§	§	§
$I \times V$	28	12						
Size (S)	1	2	§	§	§	§	§	§
$V \times S$	7	3	§	§	§	§	NS	NS
$I \times S$	4	4	NS	NS	NS	NS	NS	NS
$I \times V \times S$ (Error)	28	12						
Totals	79	59						
R ² (%)			99.0	99.1	98.9	98.4	94.9	95.2
RMSE (% pts)#			0.22	0.24	0.15	0.17	0.16	0.16

* Degrees of freedom

† Eight varieties, two sizes

‡ Four varieties, three sizes

§ Significant at $P > 0.0001$

|| Significant at $0.0001 < P < 0.05$

Root mean square error of ANOVA table

discrepancies (in trade) even if instrument brands are fundamentally accurate relative to the laboratory (over many samples). However, seed density was not producing any consistent pattern of test results.

MIXED-VARIETY SOYBEAN SET

The physical properties of the state samples are shown by size in figure 2. Test weight and density decreased with increasing size and seed weight. These were consistent with the single-variety results.

Figure 3 shows the instrument data for protein and oil, by size. Seven states had samples large enough in four sizes; 16 states had samples large enough in three sizes. In both instances, the patterns repeated those of the single-variety set.

CORRELATIONS AMONG SOYBEAN DATA

There were few significant interrelationships among the quality factors (table 6). Test weight was not a conclusive

Table 4. Properties of the sized soybean samples, single-variety set*

	Eight Samples, Two Sizes by Size (64th in.) (mm)		Four Samples†, Three Sizes by Size (64th in.) (mm)		
	(5.6-6.4)	(6.4-7.2)	(5.6-6.4)	(6.4-7.2)	(7.2-8.0)
	14-16	16-18	14-16	16-18	18-20
Oven moisture (%)	9.2 ^A ‡ (0.9)	9.4 ^A (0.9)	9.6 ^C (1.1)	10.0 ^C (0.9)	9.4 ^C (0.6)
Protein (%)§	35.6 ^A (1.3)	36.1 ^B (1.5)	35.2 ^C (1.8)	35.4 ^C (1.9)	36.2 ^D (1.8)
Oil (%)§	17.0 ^A (0.7)	17.4 ^B (0.7)	17.2 ^C (0.8)	17.8 ^D (0.6)	17.7 ^D (0.6)
Fiber (%)§	4.8 ^A (0.4)	4.6 ^B (0.2)	5.0 ^C (0.4)	4.6 ^D (0.2)	4.4 ^E (0.1)
Test Weight (lb/bu)	56.2 ^A (0.7)	56.2 ^A (0.8)	56.2 ^C (0.9)	56.3 ^C (1.1)	55.6 ^D (1.3)
Density (g/cm ³)	1.246 (0.010)	1.245 (0.013)	1.240 (0.011)	1.237 (0.007)	1.238 (0.008)

* Standard deviation in parenthesis.

† Century 84, DeKalb 174, Elgin, Unknown.

‡ Values within a sample-size group with the same letter are not significantly different ($P = 0.05$)

§ Laboratory chemistry done by Woodson-Tenant, Inc., basis 13.0% moisture

|| Basis 13.0% moisture

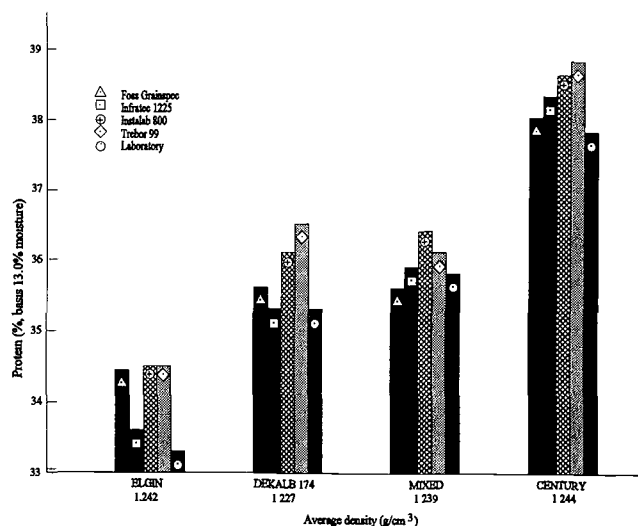


Figure 1—Soybean protein predictions, by variety, three sizes combined.

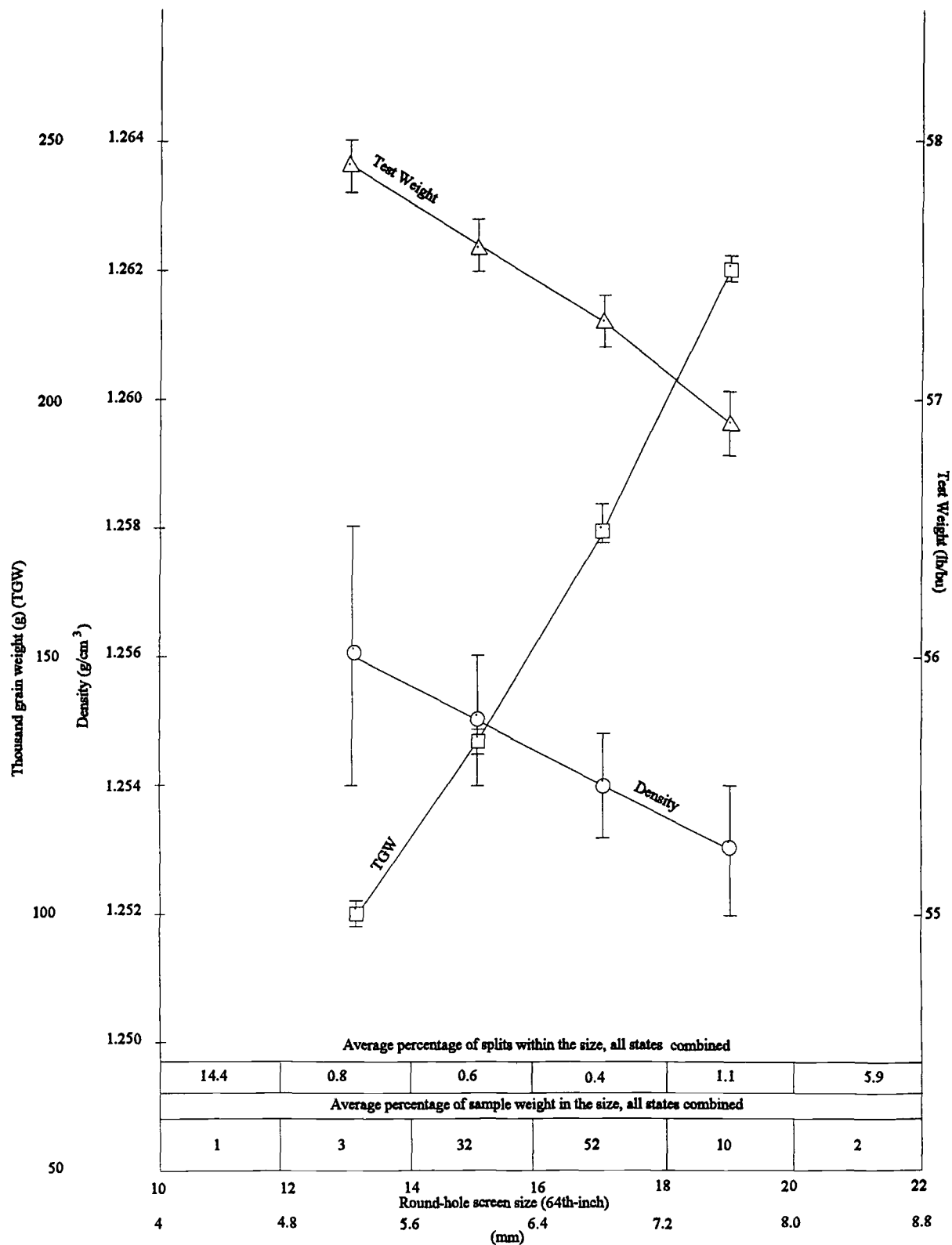


Figure 2—Physical properties of state-composite soybean samples (by seed size).

measure of density. The negative relationship between oil and protein was expected; but the general change in constituents across sizes damped out what normally is a greater-than-0.9 correlation. The negative correlation between density and oil was also expected because oil has

a specific gravity less than 1.0, while organic matter is usually 1.2 to 1.4. Seed weight, size held constant, had no effect on any other property. Density was not correlated with any whole-grain instrument differences with either chemistry or the ground-grain analyzer.

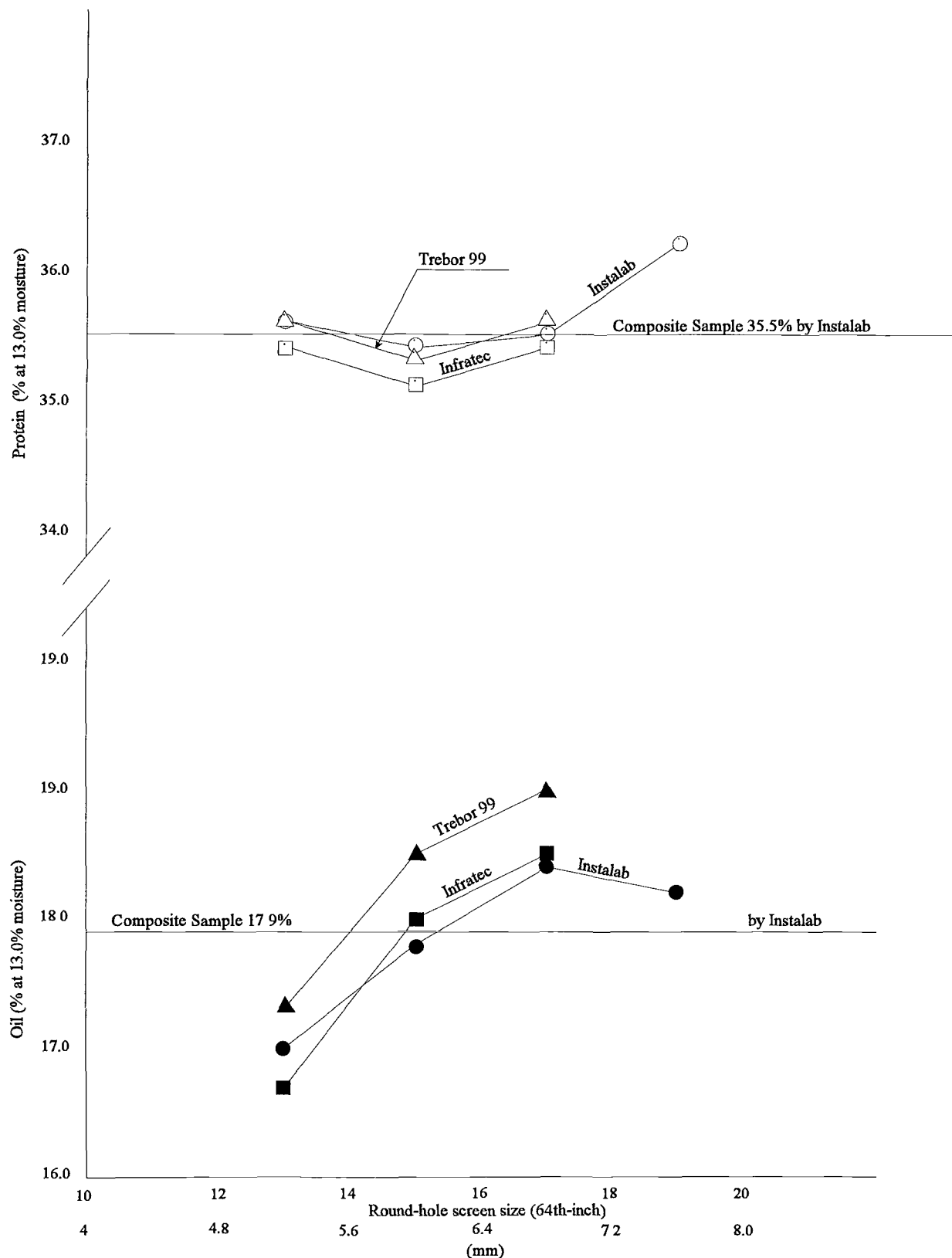


Figure 3—Protein and oil predictions (by seed size and instrument). Four varieties, three sizes.

CORN

The properties of the sized, weight-separated samples are given in table 7. The superscript letters indicate statistical equivalence ($P = 0.05$) as given by the analysis of variance, within a row (across treatments).

There was a clear separation between the sizes in seed weight and protein content. Interestingly, the thins were lower in protein, which supported the dry millers' belief that thins produce poorer quality flaking grits as well as smaller grits. The aspiration sorted for test weight and

Table 6. Correlations (r) among soybean quality factors (by size)

Factor Pair	Size Range, 64th in. (mm)				
	(4.8-5.6)	(5.6-6.4)	(6.4-7.2)	(7.2-8.0)	All
	12-14 (S,M)†	14-16 (S,M)	16-18 (S,M)	18-20 (S,M)	(S,M)
Oil - Test weight	N/A‡, NS§	NS, NS	NS, NS	NS, NS	-0.65, -0.71
Density - Test weight	N/A, 0.63	0.64, 0.57	NS, 0.65	NS, 0.50	0.54, 0.78
Oil - Protein	N/A, -0.64	-0.75, -0.56	-0.80, -0.85	-0.94, -0.81	-0.64, -0.75
Density - Oil	N/A, -0.83	NS, -0.67	NS, -0.62	NS, -0.78	-0.56, -0.68

* All r significant at P = 0.05.

† S = single variety set; M = mixed variety set.

‡ Not applicable (samples too small).

§ Not significant.

density, but did not sort by composition. Neither the sizing nor the weight separation affected the comparability of the two instruments. The protein range for these samples was 7.0 to 9.9% and the oil range was 3.2 to 4.3% (both basis 15% moisture).

Because the sizing was not related to NIR-NIT accuracy, all data was pooled for a stepwise regression for protein difference (NIT-NIR) against seed properties. A two-factor model was significant (both factors above the 0.05 level):

$$DP = -9.0 + 6.27 D + 0.00166 \text{ TGW}$$

$$R^2 = 33\%, \text{ Standard deviation} = 0.25\% \text{ points}$$

where

DP = NIR protein minus NIT protein (% points, basis 15% moisture)

D = Density (g/cm³, basis 15% moisture)

TGW = Thousand seed weight (g basis 15% moisture)

Over the range of densities in this data set, the maximum density impact on protein prediction was ± 0.12 percentage points.

Recent work at Iowa State had indicated that both the NIR and NIT analyzers can predict corn seed density with $R^2 > 90\%$ (Siska and Hurburgh, 1993). The net effect is

that near-infrared analyzers do need a corn calibration set robust in density.

The only significant correlations among properties (consistent across all sizes and weights) were test weight-density ($r = 0.67$) protein-starch ($r = -0.87$), and density-protein ($r = 0.62$). Test weight is a measure of bulk density, which is partly determined by seed density. Protein and starch are normally inversely related in corn genetics, because dense corn contains more hard endosperm, which is relatively higher in protein.

CONCLUSIONS

- Neither soybean seed size, nor seed density influenced the accuracy of protein and oil predictions by whole-grain near-infrared transmission.
- With increasing soybean seed size from 12/64 in. (4.8 mm) round to 20/64 in. (8.0 mm) round:
 - Protein content increased approximately 1 percentage point.
 - Oil content increased approximately 1.3 percentage points up to 18/64 in. (7.2 mm), then held constant or decreased slightly.
 - Test weight and density decreased by one lb/bu and 0.003 g/cm³, respectively. Seed weight doubled.
 - Fiber content decreased about 0.5 percentage points.
- Corn seed size did not affect the accuracy of whole grain near-infrared transmission analyzers.
- Corn seed weight and seed density were weakly related ($R^2 < 35\%$) to near-infrared transmission analyzer accuracy, with practical effects in protein of less than $\pm 0.2\%$ points.
- Inclusion of seed density and weight as sample selection variables in corn calibration sets could eliminate these weak effects as well as support the documented ability of near-infrared analyzers to measure corn density.

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Table 7. Properties of the sized corn samples

Property	Thins (< 20/64 in. 8.0 mm)			Large (> 20/64 in. 8.0 mm)		
	Heavy	Combined	Light	Heavy	Combined	Light
Test weight (lb/bu)	58.3A*	57.2a	56.1B	58.2A	57.4A	56.6C
Density (g/cm ³)	1.264A	1.263A	1.261B	1.271C	1.268B	1.266A
Instalab density (g/cm ³)	1.257A	1.253A	1.250B	1.266C	1.264B	1.262D
Seed weight (g/1000 seeds)	285A	276A	268B	357C	345B	332D
Instalab protein (%)	8.1A	8.1A	8.1A	8.5B	8.5B	8.4B
Infratec protein (%)	8.2A	8.2A	8.2A	8.6B	8.5B	8.4B
Protein differenceNS† (% points)	-0.1	-0.1	-0.1	-0.1	0.0	0.1
Instalab oil (%)	3.7A	3.7A	3.7A	3.5B	3.5B	3.5B
Infratec oil (%)	3.7A	3.7	3.7A	3.6B	3.6	3.6B
Oil differenceNS (% points)	0.0	0.0	0.0	-0.1	-0.1	-0.1
Instalab Starch (%)NS	60.5	60.5	60.4	60.2	60.3	60.4

* Means on the same row with same letter were equivalent (P = 0.05) in the analysis of variance

† No treatment effect was significant

NOTE: Basis 15% moisture

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